

## Mapping of a Gene for Autosomal Dominant Juvenile-Onset Open-Angle Glaucoma to Chromosome 1q

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### Summary

A large Caucasian family is presented, in which a juvenile-onset form of open-angle glaucoma is transmitted in an autosomal dominant fashion. Sixteen affected family members were identified from 31 at-risk individuals descended from the affected founder. Affected patients developed high intraocular pressures (sometimes >40 mm Hg) within the first 2 decades of life. Linkage analysis between the disease phenotype and 12 microsatellite repeat markers located on chromosome 1q gave a maximum lod score of 8.38 at a recombination fraction of zero for marker D1S210. Analysis of recombinant haplotypes suggests a total inclusion region of about 14 cM between markers D1S194 and D1S218 at 1q21–q31. This represents the second juvenile-glaucoma family, in which the disease has been mapped to the long arm of chromosome 1.

### Introduction

Glaucoma, a leading cause of blindness worldwide, is a term that encompasses a group of ocular diseases in which elevated intraocular pressure is usually present. The natural history of the disease is damage to the optic nerve, resulting in loss of the visual field, which can lead to blindness. Primary open-angle glaucoma (MIM 137760; McKusick 1992) is the most common form of glaucoma and is characterized by onset in middle age or later, elevated intraocular pressure, an open, normal appearing anterior chamber angle, and damage to the optic nerve and visual field. Family history is one of the risk factors for glaucoma, but the relatively late age at onset, common to most forms of open-angle glaucoma, complicates efforts to identify a clear-cut mode(s) of inheritance (Wilson et al. 1987). Juvenile glaucoma (MIM 137750; McKusick 1992) occurs during late childhood and early adulthood (Ellis 1948; Francois 1980; Valtot et al. 1991) and is more frequent among Americans of African ancestry (Lotufo et al. 1989;

Sommer et al. 1991; Tielsch et al. 1991). It can occur with abnormalities of the irides or anterior chamber angle (Berg 1932; Weatherill and Hart 1969; Martin and Zorab 1974) or without them (Courtney and Hill 1931; Stokes 1940). Many juvenile-glaucoma families have been reported as showing autosomal dominant inheritance, although instances of recessive inheritance also have been reported (Bell 1932; Stokes 1940; Beiguelman and Prado 1963; Crombie and Cullen 1964).

Johnson et al. (1993) described a family in which juvenile-onset open-angle glaucoma was inherited in an autosomal dominant fashion. The disease state in affected family members included onset during the first 3 decades of life, normal anterior chamber angles, high intraocular pressures, lack of systemic or other ocular abnormalities, and need for surgery to control the glaucoma in affected individuals. The disease was subsequently linked to markers in the 1q21–q31 region (Sheffield et al. 1993).

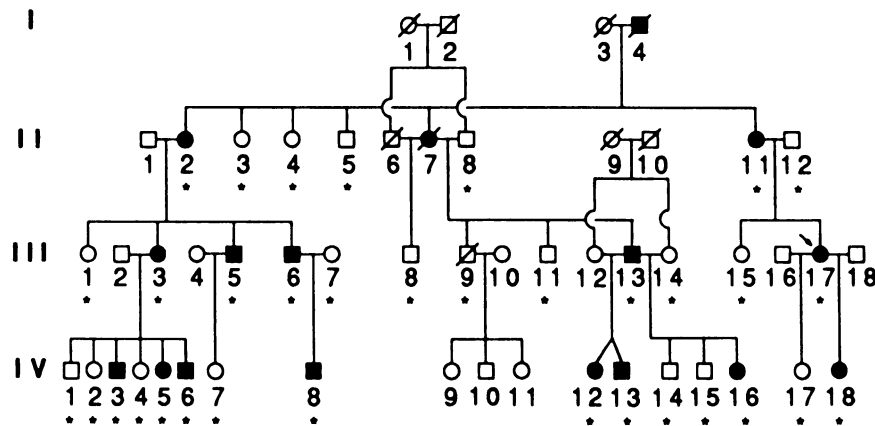
In this paper, we present clinical data and genetic analysis of autosomal dominant juvenile-onset open-angle glaucoma in a large Caucasian family different from the family reported by Johnson et al. (1993) and Sheffield et al. (1993). We present linkage data for 12 microsatellite repeat markers from the chromosome region 1q21–q44. Several genes previously mapped to this region are discussed as possible candidate genes.

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# UM:JG-1 1989



**Figure 1** UM:JG-1 pedigree. The pedigree presented here contains all of the individuals used in the linkage analysis. No blood collection or DNA analysis was carried out for deceased individuals, marked with a diagonal line. Squares are males; circles are females. Unblackened symbols represent unaffected family members; blackened symbols represent affected family members. \* = Individuals for whom diagnosis originates directly from an ophthalmologist. The arrow indicates the proband (III-17).

## Subjects and Methods

### Family and Patients

Thirty individuals in family UM:JG-1 (fig. 1), including spouses of affected family members, were examined at the University of Michigan W. K. Kellogg Eye Center. One of us (P.R.L.) examined 11 of the 17 affected family members, 13 of the at-risk but unaffected members of the pedigree, and 4 spouses of affected family members. Clinical phenotypes for the remaining family members, including spouses of some affected family members, were assigned by ophthalmologists elsewhere (five subjects), by reports of other family members (six subjects), or by self-report (nine subjects). Affected status was established by complete ophthalmologic examination including measurement of intraocular pressure, slit-lamp biomicroscopy, gonioscopy, and optic disk examination. Criteria for diagnosing glaucoma were (1) intraocular pressures >20 mm Hg, with characteristic optic disk and/or visual field damage or (2), in the absence of optic disk and visual field damage, an intraocular pressure >30 mm Hg.

Twenty to 40 ml of whole blood were collected in EDTA and used for DNA extraction and preparation of transformed cell lines (Kunkel et al. 1982). Lymphocytes were transformed with Epstein-Barr virus, in the presence of conA (Miller and Lipman 1973; Anderson and Gusella 1984). DNA and transformed cell lines

were prepared from 15 affecteds, 14 at-risk unaffecteds, 9 spouses of affecteds, and the spouse and unaffected children of 1 deceased at-risk unaffected.

All genetic markers used in these experiments were microsatellite repeats assayed by PCR (Mullis and Faloona 1987; Litt and Luty 1989; Weber and May 1989). Marker locations, primer sequences, and PIC for each marker are provided in table 1. For each marker assay, one of each primer pair was <sup>32</sup>P end-labeled (Richardson 1981). PCR reactions contained 0.025 U *Taq* polymerase in 0–25-μl vol containing end-labeled primer at 0.2 ng/μl plus the same primer unlabeled at 0.2 ng/μl, the unlabeled paired primer at 0.4 ng/μl, 3 mM MgCl<sub>2</sub>, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 50 mM KCl, and 10 mM Tris-Cl pH 9.0 (25°C). Conditions for thermal cycling were as follows: one round at 94°C for 5 min, followed by 29 cycles (94°C, 10 s; 55°C, 10 s; and 74°C, 30 s), followed by 10 min at 74°C, and storage at 4°C. The resulting PCR products were analyzed by size fractionation on 6% denaturing acrylamide gels, followed by autoradiography of the dried gel. pBlue-script plasmid DNA sequence generated by dideoxynucleotide partial chain termination sequencing reaction was used as size standard for determination of allele sizes (GenBank X52324 and X52329; Sanger et al. 1977; Tabor and Richardson 1987; Altling-Mees and Short 1989).

**Table 1****Chromosome 1 Microsatellite Repeat Markers**

Locus	Location	PIC	Reference	Primer Sequences
APOA2 .....	1q21-q23	.70	Weber and May 1989	{GAT TCA CTG CTG TGG ACC CA GGT CTG GAA GTA CTG AGA AA
D1S103 .....	1q32-q44	.80	Weber et al. 1990a	{ACG AAC ATT CTA CAA GTT AC TTT CAG AGA AAC TGA CCT GT
D1S104 .....	1q21-q23	.70	Weber et al. 1990b	{ATC CTG CCC TTA TGG AGT GC CCC ACT CCT CTG TCA TTG TA
D1S191 .....	1	.75	Weissenbach et al. 1992	{CCA CTG TTC TGC TTG AAG GT ATG CAT TTG CTT ACA AAT ATC C
D1S194 .....	1	.67	Weissenbach et al. 1992	{AGC TAG GCT GTA AGT TTC TGC TC GTC TCT TGC TGG ACT GGG A
D1S210 .....	1	.65	Weissenbach et al. 1992	{CCT CAG TTC ATT CCC CAT AA AGC TGA ATC TCA CCC AAT AAC TA
D1S212 .....	1	.80	Weissenbach et al. 1992	{CAG CAA GAC TCT GCC TCT AC CCA GGC TGA TTT TGT GTA TG
D1S215 .....	1	.73	Weissenbach et al. 1992	{GAC ACA GGT AGG TTA GAA GGA TG TGT CTT GGT GAA TTG ACC CT
D1S218 .....	1	.84	Weissenbach et al. 1992	{TGT AAA AGC AAA CTG TAG ACG AT TTT ATG TTA TCA CCA AGG CTT CT
D1S240 .....	1	.63	Weissenbach et al. 1992	{ATT GCA GTG ATT GTG GAA C TAG GAA CTT AGG GTG GTC AT
D1S306 .....	1	.62	Weissenbach et al. 1992	{CTG GGA CTG GAA ACA CTT TTG AT CCA GAG GGA GCA TTG GTG
LAMB2 .....	1q31	.88	Engelstein et al. 1993	{CTC GAA AGC TTG CTG AAC GG GTG CAA AGT GGG TTA TGT AGA AGT

Linkage analysis was performed by the method of lod scores (Morton 1955). Calculations were carried out with the program MENDEL (Lange et al. 1988). The lod score  $Z(\theta) = \log_{10}[L(\theta)/L(1/2)]$ , where  $L(\theta)$  is the likelihood of the linkage data for recombination fraction  $\theta$ . The estimate of the recombination fraction  $\hat{\theta}$  is obtained by maximizing the likelihood or, equivalently, the lod score. A maximum lod score  $Z(\theta) \geq 3.0$  is generally accepted as conclusive evidence in favor of linkage, while a lod score  $Z(\theta) \leq -2.0$  is generally accepted as excluding linkage up to that recombination fraction (Morton 1955).  $\theta(-2)$  is the recombination fraction at which the lod score equals  $-2$ , so that smaller recombination fractions can be excluded.

Lod score calculations made use of data for all family members except unaffected individuals <17 years of age. The assumption was made that no sporadic cases occurred. The analysis was repeated with unaffected family members coded as unknown, to obtain an estimate of the evidence for linkage that was independent of any (possibly incorrect) penetrance assumption. Cal-

culations were carried out, with allele frequencies for the dominant juvenile-glaucoma allele set equal to .001 or .00001. The true frequency of this allele is unknown but is believed to be between these two values. Marker-allele frequencies for this analysis were estimated from this pedigree by maximum likelihood (Boehnke 1991); estimates observed were compared with marker-allele frequencies obtained from Genome Database (Johns Hopkins University) and were found to be in good agreement. To investigate the robustness of the linkage analysis to misspecification of the allele frequencies, the lod score calculations were repeated assuming equal frequencies of the marker alleles observed in the pedigree.

## Results

As shown in figure 1, the glaucoma in this family appears to be transmitted in an autosomal dominant fashion. Of the 31 individuals at risk, 16 (52%) are affected. The founder (I-4) is not considered at risk, since

diagnostic information on his parents is not available, although the disease is reported among some of his earlier relatives. Age at diagnosis averaged 11.5 years. The only demonstrated case of detection of glaucoma beyond the age of 17 years occurred in individual II-2, who was already severely affected by the time of diagnosis at age 27 years. Intraocular pressures in affected family members were high, frequently reaching 40–50 mm Hg (normal intraocular pressure is 10–20 mm Hg). The clinical phenotype in this family is characteristic enough that young members of UM:JG-1 who appear with pressures >30 mm Hg are classified as affected and receive immediate filtering surgery, whether or not there is glaucomatous optic disc and visual field damage. Experience with numerous members of this family has shown that medical management of this disease is not adequate to reduce intraocular pressure to a level low enough to prevent damage from occurring and/or progressing. Although iris processes were observed in the anterior chamber angle of some family members, they were not consistently associated with either the presence or the absence of glaucoma. No difference in the pattern of refractive error was noted between affected and unaffected individuals.

Linkage analysis of data obtained from 57 microsatellite repeat markers from 20 autosomal chromosomes confirmed that members of this family are related as purported by family history. Analysis of 45 markers not on chromosome 1 excluded 20%–25% of the autosomal genome (data not shown). Karyotype of the affected proband was normal (data not shown).

For two different markers, single instances of incompatibility were observed in which a child presented with an allele not observed in the parents, even though the parents were confirmed as the child's parents by analysis of all other markers. Each case involved a different individual. A second experiment confirmed the incompatibility in both cases. These data suggest a possible mutation rate for microsatellite repeat markers of about 0.1%, consistent with the findings of Weissenbach et al. (1992). This is a minimum estimate, since our data set may contain additional mutations in which an allele was mutated to a different size also present in the parents, resulting in apparent compatibility. Haplotype analysis of the chromosome 1 markers suggests that this is probably not the case for any of the allele assignments used to define the interval likely to contain the gene.

Statistically significant evidence for linkage to chromosome 1q was found for six markers, when data were analyzed for all individuals, with phenotypes assigned

as indicated in figure 1 (table 2). Evidence for linkage to chromosome 1q was substantially reduced but still strong when linkage analysis was performed with the phenotype of unaffected individuals assumed unknown (table 3). The maximum lod score was obtained for marker D1S210 ( $\bar{Z} = 8.38$  at  $\theta = .00$ ) (table 2). Lod scores for the other markers shown in tables 2 and 3 establish a probable inclusion region, within which the gene is likely to be located, of about 25 cM when unaffecteds are left unspecified in the analysis, and about 14 cM when all of the phenotypic assignments shown in figure 1 are used. Marker D1S196, which was shown to be linked to the disease in the family of Sheffield et al. (1993), was uninformative in this family (data not shown). Varying the disease-allele frequency from .001 to .00001 resulted in no detectable change in the outcome of the analysis. Allele frequencies in tables 2 and 3 were generated by maximum-likelihood analysis of the family (Boehnke 1991). Essentially the same results were obtained by the use of published allele frequencies or by the assignment of equal frequencies for all alleles.

Specific recombination events detected in individual family members further aided in localizing the most probable location for the gene. Where possible, the allele assignments and haplotypes for deceased individuals were reconstructed on the basis of the genotypes of their children and, in some cases, spouse. As illustrated in figure 2, haplotype analysis of individuals III-3, IV-4, and II-3 suggests a location distal to D1S194; that of individual III-1 suggests a location proximal to D1S218; that of individual III-11 suggests a location proximal to D1S212; that of individuals IV-1 and II-7 suggests a location proximal to D1S238; and that of individuals IV-6, III-6, and IV-18 suggests a location proximal to D1S306 (data not shown). No recombination events were observed for marker D1S210.

The most likely order and spacing of markers, derived from UM:JG-1 data by minimization of the sum of the adjacent recombination fractions (see Olson and Boehnke 1990), are D1S104–(5 cM)–D1S194–(11 cM)–D1S210–(2 cM)–D1S218–(4 cM)–D1S212–(2 cM)–D1S215–(3 cM)–LAMB2–(4 cM)–D1S191, D1S240–(2 cM)–D1S238–(11 cM)–D1S306. This provides previously unreported distances between D1S104 and D1S194 and places LAMB2 relative to the other markers. This is the same order reported by Weissenbach et al. (1992), except for placement of LAMB2, which does not appear on their map. They use D1S104 (1q21–q31) to anchor their data for this region to other maps, without providing a specific distance between D1S104 and D1S194.

**Table 2****Lod Scores for Complete Penetrance**

MARKER	LOD SCORE AT $\theta =$						$\hat{\theta}$	$\hat{Z}$	$\theta(-2)$
	.01	.05	.10	.20	.30	.40			
D1S104 .....	-5.23	-1.35	.04	.95	1.01	.65	.26	1.05	.03
D1S194 .....	-.17	1.66	2.18	2.20	1.68	.87	.15	2.28	.00
D1S210 .....	8.25	7.71	7.01	5.48	3.76	1.80	.00	8.38	.00
D1S218 .....	5.41	5.63	5.30	4.24	2.91	1.39	.04	5.66	.00
D1S212 .....	2.11	3.14	3.27	2.82	1.98	.91	.09	3.27	.00
D1S215 .....	1.16	2.24	2.42	2.11	1.46	.65	.10	2.42	.00
LAMB2 .....	2.49	2.85	2.72	2.11	1.31	.46	.05	2.85	.00
D1S240 .....	2.47	3.49	3.60	3.14	2.29	1.18	.09	3.61	.00
D1S191 .....	3.05	4.02	4.06	3.45	2.42	1.15	.08	4.09	.00
D1S238 .....	.02	2.37	2.98	2.91	2.18	1.10	.14	3.08	.00
D1S306 .....	-6.11	-2.22	-.80	.18	.35	.22	.29	.35	.05
D1S103 .....	-21.34	-11.14	-6.99	-3.26	-1.46	-.48	.50	.00	.25

**Discussion**

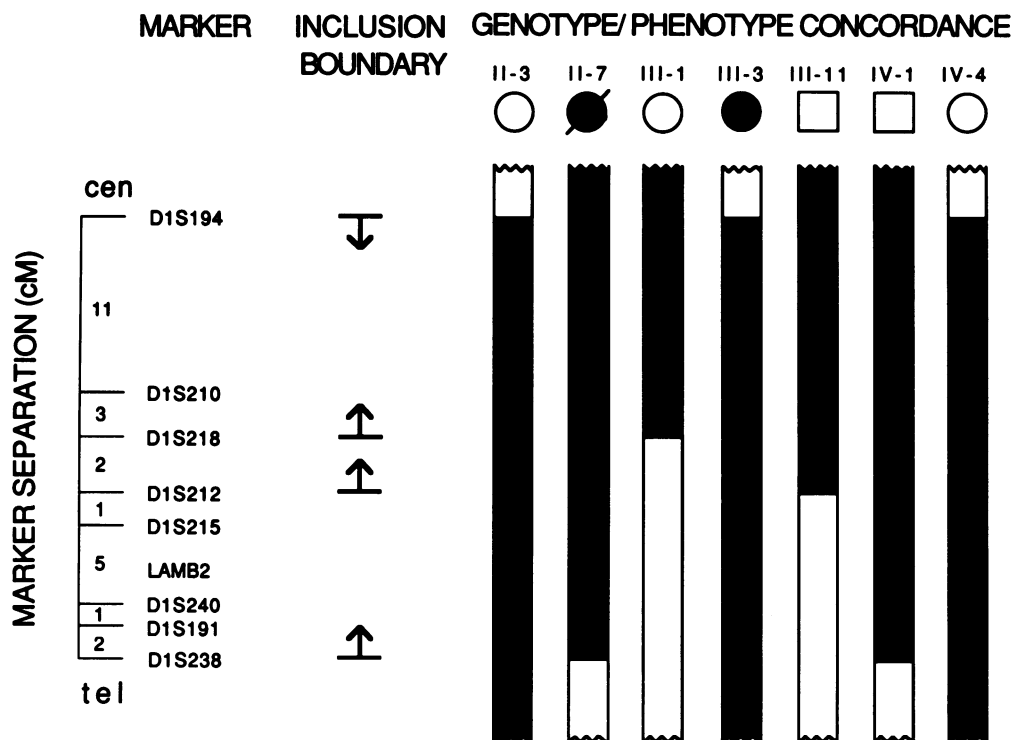
The gene responsible for autosomal dominant juvenile-onset open-angle glaucoma in this family is linked to D1S210 and surrounding markers on chromosome 1q. By evaluating recombination events detected in affected individuals only, the interval within which the gene likely resides is  $\leq 25$  cM, flanked by proximal marker D1S194 ( $\hat{Z} = 2.03$  at  $\hat{\theta} = .07$  with affected recombinant III-3) and distal marker D1S238 ( $\hat{Z} = 2.32$  at  $\hat{\theta} = .07$  with affected recombinant II-7) (table 3). The best estimate, based on analysis of both affected and unaffected individuals, is that the gene is contained within a region of about 14 cM containing D1S210 and

flanked by markers D1S194 ( $\hat{Z} = 2.28$  at  $\hat{\theta} = .15$ ) and D1S218 ( $\hat{Z} = 5.66$  at  $\hat{\theta} = .04$ ). The assignment of the proximal boundary at D1S194 is supported by recombination events in both affected and unaffected individuals. Assignment of this distal boundary at D1S218 depends on one recombination event observed in an unaffected 51-year-old individual (III-1) diagnosed by one of us (P.R.L.).

The primary issue in assignment of the distal boundary is the one of possible incomplete penetrance. In unaffected individuals, the key recombination events proximal to D1S238 occur in III-1 (recombinant at D1S218) and III-11 (recombinant at D1S212), who do

**Table 3****Lod Scores for Affected Individuals Only**

MARKER	LOD SCORE AT $\theta =$						$\hat{\theta}$	$\hat{Z}$	$\theta(-2)$
	.01	.05	.10	.20	.30	.40			
D1S104 .....	-1.18	-.30	.22	.58	.56	.36	.24	.60	.00
D1S194 .....	1.60	2.01	2.00	1.65	1.12	.55	.07	2.03	.00
D1S210 .....	4.11	3.82	3.45	2.64	1.75	.82	.00	4.18	.00
D1S218 .....	3.81	3.54	3.19	2.43	1.60	.74	.00	3.88	.00
D1S212 .....	2.93	2.71	2.43	1.83	1.17	.50	.00	2.98	.00
D1S215 .....	2.63	2.44	2.18	1.63	1.03	.43	.00	2.68	.00
LAMB2 .....	2.62	2.39	2.09	1.46	.81	.25	.00	2.68	.00
D1S240 .....	3.25	3.03	2.74	2.12	1.43	.68	.00	3.31	.00
D1S191 .....	3.22	2.99	2.68	2.03	1.31	.57	.00	3.28	.00
D1S238 .....	1.86	2.30	2.29	1.93	1.36	.67	.07	2.32	.00
D1S306 .....	-3.57	-2.06	-1.20	-.41	-.08	.02	.43	.03	.05
D1S103 .....	-4.66	-3.77	-2.74	-1.37	-.63	-.23	.50	.00	.14



**Figure 2** Region of genotype/phenotype concordance expected to contain the juvenile-onset glaucoma gene. A diagonal line indicates that the individual is deceased and that the genotype was reconstructed on the basis the genotypes of relatives, including children and spouse of the deceased individual. Vertical bars are the region of chromosome 1 corresponding to the markers (*left*). For each marker, haplotype analysis was carried out, with assignment of alleles as cotransmitting with the disease or normal phenotypes. Blackened regions of a bar indicate the region in which the allele scored corresponds with phenotype of the individual. For example, individual III-1 has a normal phenotype and carries alleles cotransmitting with the disease between D1S218 and D1S238, so this region is unblackened to indicate discordance of genotype and phenotype. Above D1S218, III-1 carries alleles that are not found to cotransmit with the disease in other family members, so the region above D1S218 is blackened to indicate that the disease cannot be at D1S218 but could be anywhere above it on this map. Published distance between markers derives from Weissenbach et al. (1992). Placement of LAMB2 between D1S215 and D1S191 derives from UM:JG-1 data (data not shown). The inclusion boundary column indicates the locations of recombination breakpoints in individuals (*right*) and the direction from that breakpoint to the gene determined by haplotype analysis.

not represent borderline diagnoses. Both are well past the 17-year age cutoff and have intraocular pressures in the low teens. In addition, individual III-1 has five unaffected children, ages 28–32 years (data not shown). If III-1 were, in fact, a case of nonpenetrance, then markers D1S210 (proximal) and D1S212 (distal) would flank a 5-cM region containing D1S218 and the disease gene. If III-11 were also a case of nonpenetrance, D1S218 (proximal) and D1S238 (distal) would flank a region of 11 cM with no recombinants at markers D1S212, D1S215, LAMB2, D1S240, and D1S191. If the phenotypic assignments were correct for III-1 and incorrect for III-11, contradictory locations would be indicated.

The 14-cM region between D1S194 and D1S218 is large enough to contain many genes. Several genes al-

ready mapped to this region have properties that recommend them as possible candidates for the juvenile-glaucoma gene. Sheffield et al. (1993) proposed the atrial natriuretic peptide receptor gene ANPRA (1q21–q22) (Lowe et al. 1990) as a candidate juvenile-glaucoma gene, in part because of its suggested role in experimental glaucoma (Fernandez-Durango et al. 1990) and the observation that intravenous administration of atrial natriuretic peptide can reduce elevated intraocular pressure in humans (Diestelhorst and Kriegelstein 1989).

The laminin B2 gene (LAMB2; 1q25–q31) (Fukushima et al. 1988) produces one of the subunits of laminin, a major component of basement membranes of the trabecular meshwork (Kurosawa et al. 1987; Murphy et al. 1987). Alteration in laminin might affect the intraocular pressure by altering the outflow facility, but altered

laminin might be expected to have additional systemic manifestations. One recombination event was observed between a microsatellite repeat marker derived from an LAMB2 clone (GenBank M55202; Engelstein et al. 1993) and the clinical phenotype in unaffected family member III-1. Because this marker is located within the gene, this recombination event alone cannot place the entire gene outside the inclusion interval. However, the marker order determined from UM:JG-1 data suggests that LAMB2 is distal to D1S215, which leaves two additional recombination events, at D1S218 and D2S212, between LAMB2 and the inclusion interval.

Two types of ATPase genes that map to 1q21–q31 have properties that suggest a possible role in regulation of intraocular pressure. ATP2B2, encoding the beta subunit of a plasma membrane  $\text{Ca}^{++}$ -transporting ATPase, maps to 1q25–q32 (Olson et al. 1991). Kobayashi et al. (1989) report  $\text{Ca}^{++}$ -transporting ATPase activity in rabbit trabecular meshwork and suggest that ATP-dependent  $\text{Ca}^{++}$  transport may affect the outflow facility by regulating contractility of the trabecular meshwork. The  $\text{Na}^{+}$ - $\text{K}^{+}$ -ATPase gene ATP1B1 (1q22–q25) (Yang-Feng et al. 1988; Lane et al. 1989; Seldin et al. 1989) is expressed in the nonpigmented ciliary epithelium of the eye and is involved in aqueous humor production (Becker 1980; Riley and Kishida 1986; Ghosh et al. 1991).  $\text{Na}^{+}$ - $\text{K}^{+}$ -ATPase inhibition has been shown to lower intraocular pressure, apparently by decreasing aqueous humor production (Podos et al. 1984; Uusitalo et al. 1985; Krupin et al. 1986). The pathogenesis of primary open-angle glaucoma is believed to involve abnormalities of aqueous outflow, but there is no direct evidence to exclude the existence of defects in aqueous production in a subset of juvenile-glaucoma patients.

Two previous reports have suggested association of the 1q21–q31 region with other forms of glaucoma. Tinning et al. (1975) reported diagnosis of congenital glaucoma at age 18 mo, in a child with a balanced translocation t(1;6)(q23;q27). Possible involvement of the 1q region in the pathogenesis of primary open-angle glaucoma was suggested by David and Jenkins's (1980) report of a weak association between the Duffy blood group (FY; 1q21–q25) (Raeymaekers et al. 1989; Rogne et al. 1989) and primary open-angle glaucoma in a Caucasian population.

Sheffield et al. (1993) reported linkage of autosomal dominant juvenile-onset glaucoma to D1S212 ( $\bar{Z} = 6.5$  at  $\hat{\theta} = .00$ ) and surrounding markers on 1q. This report describes a different family with the same phenotype that maps to this region. The finding of linkage to the same location suggests that the same gene plays a role in

the disease process in both families. The clinical phenotype in the two families is similar in many ways, including the high intraocular pressures observed (Johnson et al. 1993).

Although the disease segregating in this family is a form of open-angle glaucoma, the age at onset is earlier than that observed for the more common primary open-angle glaucoma. This form of juvenile-onset glaucoma is characterized by intraocular pressures up to 40–60 mm Hg, which is higher than that usually observed for primary open-angle glaucoma. Whether this entity is genetically distinct from primary open-angle glaucoma remains to be established.

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